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Detection of human muscle glycogen by natural abundance ^{13}C NMR

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ABSTRACT **Natural abundance ^{13}C nuclear magnetic resonance spectroscopy was used to detect signals from glycogen in the human gastrocnemius muscle. The reproducibility of the measurement was demonstrated, and the ability to detect dynamic changes was confirmed by measuring a decrease in muscle glycogen levels after exercise and its subsequent repletion. Single frequency gated ^{1}H decoupling was used to obtain decoupled natural abundance ^{13}C NMR spectra of the C-1 position of muscle glycogen.**

In this communication we report the observation of glycogen in the human muscle by natural abundance ^{13}C nuclear magnetic resonance. This carbohydrate storage compound is one of the principal substrates of muscle metabolism during exercise. Its concentration in human skeletal muscle is generally 70–110 mmol of glucosyl units per kg of wet tissue (1–8) and its depletion has been proposed as one of the causes of muscle fatigue following prolonged exercise (5–7).

Human muscle glycogen content is generally determined by needle biopsy (9), a procedure that involves discomfort to the subject and can only be repeated a limited number of times. Furthermore, the needle biopsy provides a measure of the glycogen content in a small volume of muscle and does not necessarily reflect the overall glycogen content of the muscle tested. In contrast, the ^{13}C NMR measurements we have made take as little as 4 min, involve no discomfort to the subject, and can be repeated as many times as the study requires. They also sample a larger volume of muscle and are therefore less susceptible to regional variations in glycogen content.

METHODS

The ^{13}C spectra were obtained at 50.4 MHz using a 4.7-T Biospec spectrometer. The homogeneous volume of the Brüker 4.7/300 horizontal magnet is centered 46 cm from the magnet endplate. It is therefore possible for most subjects to lie with one leg sufficiently far into the magnet to position the bulk of the gastrocnemius muscle in this volume. The NMR probe consisted of concentric ^{13}C and ^{1}H surface coils. The inner ^{13}C coil had a diameter of 4.5 cm and was double balanced to reduce capacitative coupling to the sample (10). The outer ^{1}H coil had a diameter of 7 cm and was of a distributed capacitance design to allow it to be tuned to 200.4 MHz. These coils were mounted below a Teflon sheet, which served as a support for the leg and also raised the leg ≈3 mm above the coils. This spacing reduced the penetration into the tissue of the high-flux regions close to the wires of the surface coils.

In this study, the ^{1}H coil was used to detect the signal from water while shimming the static magnetic field. To ensure that the correct volume of tissue was shimmed, the ^{1}H pulse width was adjusted to give maximum signal from water ≈2 cm away from the plane of the ^{1}H and ^{13}C surface coils.

The ^{13}C pulse width was calibrated by using a 1-cm-radius glass sphere containing 100 mM [1-^{13}C]glucose (99% enriched) in normal saline centered on the axis 1 cm below the plane of the ^{13}C coil. When the carrier frequency was close to the glucose C-1 resonances, the maximum signal from this sample, with a leg in position above the coil, was obtained with a 105-μs pulse of 75 W peak power. Since this corresponded to an approximate 90° pulse 1 cm from the coil, the pulse duration was increased to 250 μs so as to deliver a 90° pulse at a depth of ≈2 cm or ≈0.9 coil radii. The 90° pulse measured for the [1-^{13}C]glucose sample was always in the range of 100 to 110 μs, so a θ pulse of 250 μs with 75 W peak power was used throughout.

In a final set of experiments, the ^{1}H coil was used to deliver a decoupling field to the C-1 proton of glycogen. The strength of the decoupling field was calibrated with the same sample of 100 mM [1-^{13}C]glucose in the same position below the coil. In this final study, an improved ^{13}C coil was used, and a θ pulse of 200 μs with 75 W peak power was used.

Spectra were collected in blocks of 4000 scans, using either a θ-AQ sequence with a total repetition time of 204.8 ms between pulses or the depth pulse sequence $\theta/3(\pm x)-\theta$-AQ (11), with a total repetition time of 205.8 ms. The rf carrier frequency was positioned close to the [1-^{13}C]glycogen frequency, and a 20-kHz sweep width was used.

RESULTS

Fig. 1 shows the natural abundance in ^{13}C NMR spectrum obtained from human gastrocnemius muscle in a normal subject. It was collected in 13 min (4000 scans) using the $\theta/3(\pm x)-\theta$-AQ depth pulse sequence. The ^{1}H-coupled signal from the ^{13}C-1 position of the glucosyl monomer of glycogen is a doublet centered at 100.5 ppm, with a ^{13}C–^{1}H coupling constant of 175 Hz. It can be seen quite clearly in the expansion of the region from 80 to 115 ppm. The glycogen resonances were generally detectable with a single 4000 scan depth pulse spectrum (13 min), with a signal/noise ratio of ≈15:1 in each of the two coupled C-1 peaks. The dominant features of the spectrum are the large signals from lipids at ≈30 ppm (—CH_2—), 60–75 ppm (glycerol—CHOR— and —CH_2OR—), ≈129 ppm (—CH=CH— and —CH=CH—CH=CH—), and ≈175 ppm (—CO_2—) (12). The intensity of these signals, which arise principally from the layer of subcutaneous fat close to the surface coil, was greatly reduced by the depth pulse sequence as compared with a simple θ-AQ sequence, while the glycogen signal suffered no such reduction in intensity. The reason for the reduction in intensity of the signals from subcutaneous fat relative to the desired ^{13}C-1 signals from glycogen is the following: the effect of the depth pulse sequence $\theta/3(\pm x)-\theta$-AQ is to suppress signals from those regions receiv-

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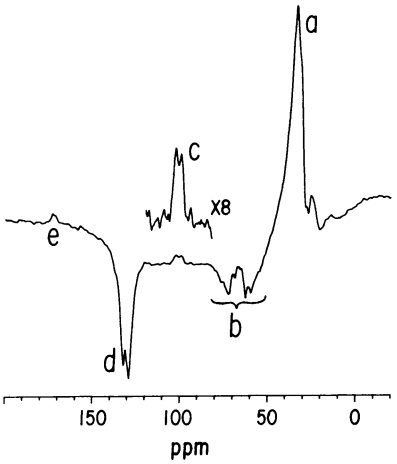

FIG. 1. Natural abundance ^{13}C NMR spectrum obtained at a field of 4.7 T, from human gastrocnemius muscle and the overlying layers of skin and subcutaneous fat. The spectrum is the sum of 4000 transients filtered with an exponential line broadening of 60 Hz prior to Fourier transformation. It was acquired with the depth pulse sequence $\theta/3(\pm x)-\theta$-AQ sequence, using a 75-W, 250-μs θ pulse and a 20-kHz sweep width. The resonances have the following assignments: a, $-CH_2-$ signals from lipids (mainly subcutaneous fat); b, —CHOR— and $-CH_2OR-$ from glycerol; c, C-1-glycogen; d, —CH=CH— and —CH=CH—CH=CH— from lipids; e, $-CO_2-$ from lipids.

ing pulses of ≈270°. The proximity of the subcutaneous fat layer to the surface coil (≈5 mm) means that when θ is optimized to deliver a 90° pulse at a depth of ≈2 cm in the gastrocnemius muscle, the volume containing the bulk of the subcutaneous fat receives pulse angles in the range of 180° to 360°. The signals from this region close to the coil are therefore suppressed, while those from the underlying muscle are relatively unaffected. This strategy considerably simplifies phasing of the C-1 glycogen signals, which in spectra obtained using a θ-AQ sequence ride on the wing of the fat signal at ≈129 ppm, with a different phase. Note that the residual signals from subcutaneous fat are ≈180° out of phase from the glycogen signals (i.e., inverted). Furthermore, the rather long θ pulse (≈250 μs) used introduces significant off-resonance effects as little as 40 ppm away from the carrier frequency, which was positioned within 5 ppm (200 Hz) of the ^{13}C-1 resonances of glycogen for all measurements.

To assess the reproducibility of the measurement, ^{13}C spectra were collected on the same individual several hours apart for comparison. The subject was removed from the magnet between measurements, but no changes were made to the spectrometer except for retuning the ^{13}C coil and reshimming the magnet at the start of each data collection. During this time, the subject undertook no strenuous exercise to ensure that there was no significant variation in muscle glycogen content. Subtraction of spectra taken as much as 24 hr apart revealed no difference in the glycogen signal intensity within the limits of the noise (Fig. 2). This corresponds to a subtraction error of ≤20%. In fact, we can better estimate the reproducibility of the glycogen measurement from the subtraction errors in the more intense lipid signals at 30 ppm and 129 ppm, since these subtraction errors are greater than the amplitude of the noise. They do represent a worst case estimate because the lipid signal arises principally from the subcutaneous fat layer, which lies closest to the coil where the B_1 field varies most rapidly with position relative to the ^{13}C coil. In contrast, the muscle mass lies deeper and sees a more uniform B_1 field. Small variations in leg position will therefore have a larger effect on the reproducibility of the lipid signal than the glycogen signal

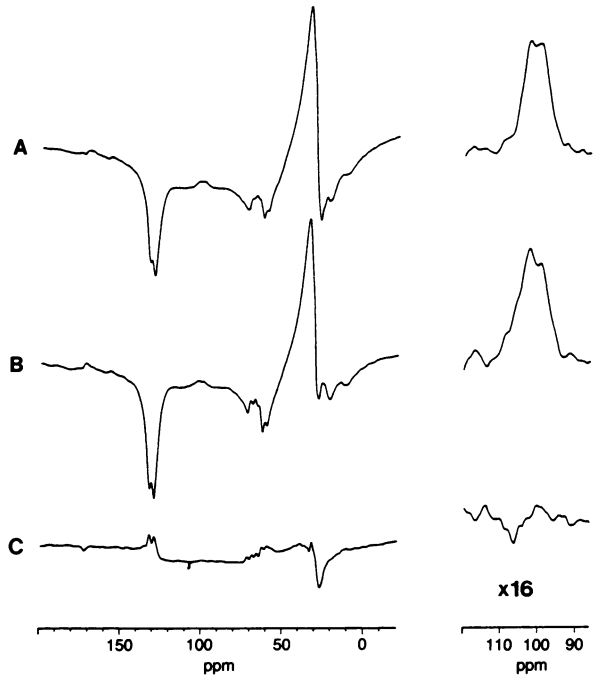

FIG. 2. Test of the reproducibility of the glycogen measurement. Spectra were collected using the depth pulse sequence $\theta(\pm x)-\theta$-AQ. Acquisition conditions were as in Fig. 1. Spectrum A was taken first, and then the subject was removed from the magnet. Spectrum B was collected the following morning, at which time the magnet was reshimmed and the ^1H and ^{13}C surface coils were retuned. Spectrum C is the difference spectrum (A − B). (*Right*) Region of these spectra around the position of the [1-^{13}C]glycogen signal expanded 16-fold. Note that the intensity of the difference peak at ≈129 ppm in spectrum C gives an indication of the reproducibility of the measurement (difference peak ≤10% of the peak in spectrum A or B).

from the deeper lying muscle. The subtraction errors in the lipid resonances at 30 ppm and 129 ppm were ≈6% and ≈12%, respectively. We therefore estimate that the error in glycogen measurement in this study from measurement to measurement is 6–10%.

To illustrate our ability to follow dynamic changes in muscle glycogen levels, the effect of strenuous exercise on calf muscle glycogen was studied in two subjects. A θ-AQ (θ = 250 μs) sequence was used to collect spectra from the gastrocnemius muscle of a trained runner prior to, immediately following, and the day after a 13-mile run (Fig. 3). Each

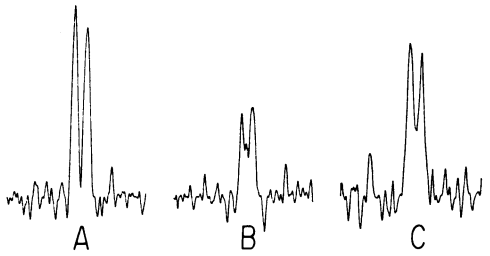

FIG. 3. The variation of the intensity of the [1-^{13}C]glycogen doublet in response to exercise. Spectrum A was collected from the gastrocnemius muscle of a trained runner 1 hr prior to a 13-mile run. Spectrum B was collected from the same location immediately following the run. Spectrum C was collected the following morning (19 hr after the run). Each spectrum is the sum of 8000 transients, filtered with an exponential line broadening of 60 Hz prior to Fourier transformation, and baseline corrected with a spline fit of the displayed region. The spectra were acquired with a θ-AQ sequence (8000 scans), using a 75-W 250-μs θ pulse and a 20-kHz sweep width.

spectrum was the sum of 8000 transients, and no adjustments were made to the spectrometer between the measurements except for reshimming the static magnetic field to optimize its homogeneity and retuning the ^{13}C surface coil. After the run, the muscle glycogen level fell to 30% of the preexercise level and had recovered to 80% of the preexercise level when measured the following morning (19 hr after the run). In a second study, the same pattern of glycogen depletion was seen—namely, the glycogen signal fell to 30% of the preexercise value following a 13-mile run. In this case, however, no glycogen measurements were performed during the subsequent recovery phase. These results are consistent with previous reports of the dynamics of the muscle glycogen pool during and after exercise. For example, Costill *et al.* (8) found that muscle glycogen fell from 2.1 per 100 g of tissue (100%) to 1.3 g per 100 g of tissue (62%) following a similar exercise regime and had increased to 1.6 g per 100 g of tissue (76%) after 24 hr (14).

The results shown thus far have demonstrated the efficacy of natural abundance ^{13}C NMR for noninvasive measurements of muscle glycogen in humans. However, ^{1}H decoupling was not used. Calculations from this laboratory indicate that single frequency ^{1}H decoupling of the [1-^{13}C]glucose doublet can be achieved without exceeding current Food and Drug Administration guidelines. Such decoupling would have the effect of doubling the signal/noise ratio of the [1-^{13}C]glycogen signal in a given time. In the final part of this study, we combined the $\theta/3(\pm x)-\theta$-AQ depth pulse sequence (θ = 200 μs) with single frequency decoupling of the C-1 proton of glycogen (5.4 ppm in the ^{1}H NMR spectrum). The decoupling field (9 W) was gated on only during the 20-ms acquisition period. This gave a 10% duty cycle for the decoupler and a mean power of 0.9 W. The results are shown in Fig. 4. The lower spectrum shows the coupled [1-^{13}C]glycogen signal obtained using the depth pulse sequence (4000 scans, 15 min), while the top spectrum, collected immediately after, shows the ^{1}H-decoupled [1-^{13}C]glycogen signal, with the expected collapse of the doublet, and consequent doubling of signal/noise ratio. This increase in signal/noise allows us to acquire useful spectra in <5 min.

We have not tried to generate a nuclear Overhauser enhancement (NOE) yet. However, if the NOE obtainable is similar to those seen at 1.9 T [1.3 ± 0.5, Neurohr *et al.* (13)] and 8.4 T [1.2, Sillerud and Shulman (14)], then an overall improvement in signal/noise of a factor of ≈2.5 can be expected. It will then be possible to make natural abundance ^{13}C NMR measurements of muscle glycogen in as little as 3 min.

In summary, these data indicate that natural abundance ^{13}C NMR spectroscopy can be used to measure routinely the levels of muscle glycogen in humans. With decoupling, the ^{13}C NMR measurement takes 5 min and, in contrast to the needle biopsy method, involves no discomfort and does not limit the number of measurements possible. Earlier work from this laboratory has shown that ^{13}C NMR detects all of the glycogen both *in vitro* and *in vivo* (14). Our tests of the reproducibility of the measurement indicate that variations in the measured glycogen intensity are no more than ≈10% at present, a level of accuracy comparable to that available with needle biopsy. The dynamic changes seen following exercise are also consistent with previous determinations of glycogen depletion during exercise and subsequent repletion.

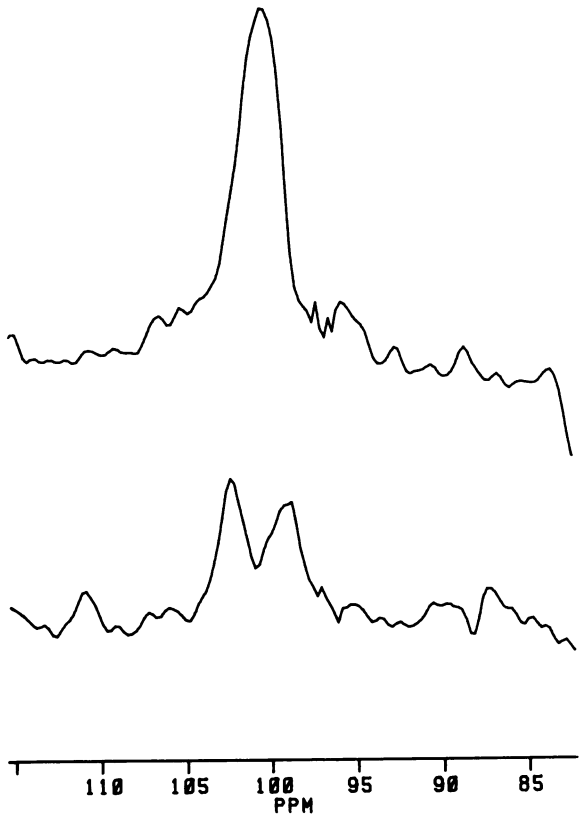

FIG. 4. The effect of single frequency ^{1}H decoupling of the C-1 proton of glycogen at 5.4 ppm in the ^{1}H spectrum. The lower spectrum was collected using the depth pulse sequence $\theta/3(\pm x)-\theta$-AQ without decoupling. The upper spectrum was collected using the same sequence but with a 9-W decoupling field on during the acquisition. With the 20-ms acquisition time and 200-ms relaxation delay, this gave a mean power deposition of 0.9 W. Both spectra were the result of 4000 scans (≈15 min), using a 75-W, 200-μs θ pulse and a 25-kHz sweep width.

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